

*J Korean Med Sci* 2009; 24 (Suppl 1): S30-7  
 ISSN 1011-8934  
 DOI: 10.3346/jkms.2009.24.S1.S30

Copyright © The Korean Academy  
 of Medical Sciences

# The Heme Oxygenase-1 Genotype is a Risk Factor to Renal Impairment of IgA Nephropathy at Diagnosis, Which is a Strong Predictor of Mortality

The induction of heme oxygenase-1 (HO-1) ameliorates oxidative stress and inflammatory process, which play important roles in IgA nephropathy. We hypothesized length polymorphism in the promoter region of the HO-1 gene, which is related to the level of gene transcription, is associated with disease severity of IgA nephropathy. The subjects comprised 916 patients with IgA nephropathy and gene data. Renal impairment was defined as an estimated glomerular filtration rate less than 60 mL/min/1.73 m<sup>2</sup> at diagnosis. The short (S: <23), medium (M: 23-28), and long (L: >28) (GT) repeats in the HO-1 gene was determined. The frequencies of S/S, S/M, M/M, S/L, L/M, and L/L genotypes were 7.2%, 6.9%, 3.1%, 30.8%, 22.7%, and 29.4%, respectively. The baseline characteristics were not different. In the S/S genotypic group, the renal impairment rate was 18.2%, which was lower than 32.2% in the group with M/M, L/M, or L/L genotype. The odds ratio of renal impairment in S/S genotype, compared to that in M/M, L/M, or L/L genotype, was 0.216 (95% confidence interval, 0.060-0.774, *p*=0.019). The HO-1 gene promoter length polymorphism was related to the renal impairment of IgA nephropathy at diagnosis, which is an important risk factor for mortality in IgA nephropathy patients.

**Key Words :** Heme Oxygenase; Glomerulonephritis, IgA; Renal Insufficiency

Ho Jun Chin<sup>\*,†</sup>, Hyun Jin Cho<sup>\*</sup>,  
 Tae Woo Lee<sup>†</sup>, Ki Young Na<sup>\*</sup>,  
 Hyung Jin Yoon<sup>‡</sup>, Dong-Wan Chae<sup>\*,†</sup>,  
 Suhnggwon Kim<sup>†</sup>, Un Sil Jeon<sup>§</sup>,  
 Jun-Young Do<sup>||</sup>, Jong-Won Park<sup>||</sup>,  
 Kyung-Woo Yoon<sup>||</sup>, Young-Tai Shin<sup>¶</sup>,  
 Kang Wook Lee<sup>¶</sup>, Ki-Ryang Na<sup>¶</sup>,  
 Dae Ryong Cha<sup>\*\*</sup>, Young Sun Kang<sup>\*\*</sup>, and  
 The Progressive REnal disease and Medical  
 Informatics and gEnomics Research (PRE-  
 MIER) members<sup>†</sup>

Department of Internal Medicine\*, Seoul National University Bundang Hospital, Seongnam; Renal Research Institute<sup>†</sup>, Seoul National University College of Medicine, Seoul; Clinical Research Institute<sup>‡</sup>, Seoul National University Hospital, Seoul; Biotechnology Center, Pohang University of Science and Technology<sup>§</sup>, Pohang; Department of Internal Medicine<sup>||</sup>; Yeungnam University College of Medicine, Daegu; Department of Internal Medicine<sup>¶</sup>, Chungnam National University College of Medicine, Daejeon; Department of Internal Medicine, Korea University Ansan Hospital<sup>\*\*</sup>, Ansan, Korea

<sup>†</sup>Members are listed in Appendix.

Received : 2 September 2008  
 Accepted : 3 November 2008

## Address for correspondence

Hyung Jin Yoon, M.D.  
 Department of Internal Medicine, Seoul National University College of Medicine, 300 Gumi-dong, Bundang-gu, Seongnam 463-707, Korea  
 Tel : +82.31-787-7025, Fax : +82.31-787-4052  
 E-mail : mednep@snuh.org

\*This study was supported by the Collaborative Research Grant of Korean Society of Nephrology.

## INTRODUCTION

Heme oxygenase (HO) is the rate-limiting enzyme for the breakdown of heme to generate carbon monoxide, iron, and biliverdin. Biliverdin is rapidly converted to bilirubin by biliverdin reductase (1). The induction of HO-1, the inducible isoform of HO, is an important endogenous mechanism for cytoprotection and mediates the beneficial effects of HO, such as antioxidant, anti-inflammatory, anti-proliferative, anti-apoptotic, and immunomodulatory effects (1). These effects may be mediated through the products of heme degradation (2-6) and regulation of MCP-1 (7), p38 mitogen acti-

vated protein (MAP) kinase (8), cell-cycle regulators (9, 10), and T-cell- and natural killer cell-mediated cytotoxicity (11).

Experimental and clinical evidences support the important protective role of HO-1 in several renal diseases. Induction of HO-1 is an adaptive and beneficial response to renal injury secondary to rhabdomyolysis (12), ischemia-reperfusion injury (13, 14), nephrotoxin (15), glomerulonephritis (16), and renal transplant rejection (17). Human patients with HO-1 deficiency showed iron deposition in the proximal tubule and pathologic change in the kidney, as well as growth retardation, anemia, leukocytosis, lymphadenopathy, and increased sensitivity to oxidative injury (18).

A (GT) $n$  repeat region located between -198 and -258 of the human HO-1 gene promoter is associated with up-regulation of HO-1 (19-21). The promoter activity of the HO-1 gene was up-regulated by H<sub>2</sub>O<sub>2</sub> exposure in genes with (GT)<sub>16</sub> or (GT)<sub>20</sub>, but not in genes with (GT)<sub>29</sub> or (GT)<sub>38</sub> (19). The reporter gene expression driven by the HO-1 gene promoter carrying (GT)<sub>22</sub> was about four- and eight-fold higher than that directed by promoters with (GT)<sub>26</sub> and (GT)<sub>30</sub>, respectively (20). Length polymorphism of this region correlates with susceptibility in several diseases, such as coronary artery disease (20, 22), ischemic cerebrovascular events (19), lung cancer (23), and renal allograft dysfunction (17, 24), although, in some reports, length polymorphism is not associated with disease conditions (25).

In IgA nephropathy, which is the most common type of primary glomerulonephritis worldwide (26), oxidative stress plays a role in the development and progression of nephropathy (27, 28). HO-1 immunoreactivity in the kidney of patients with IgA nephropathy was increased compared that in controls (27). The advanced oxidation protein products were the independent risk factor to renal progression of IgA nephropathy (27). We hypothesized that length polymorphism in the HO-1 gene promoter region will be associated with disease severity of IgA nephropathy at diagnosis.

## MATERIALS AND METHODS

### Study subjects

This study was approved by the Institutional Review Boards at the participating hospitals before the data were gathered. Informed consent was obtained from all patients. The subjects were enrolled in the Progressive RENal disease and Medical Informatics and gEnomics Research (PREMIER) study which was sponsored by the Korean Society of Nephrology. In the PREMIER study, 34 hospitals and clinics in Korea participated and shared the clinical data and genomic DNA extracted from the peripheral blood of patients with primary and secondary glomerulonephritis diagnosed by renal biopsy in each institute. There were 968 patients aged 18 yr or more with IgA nephropathy, which was diagnosed by the pathologic findings of prominent deposition of IgA antibodies in the mesangium detected by immunofluorescence staining (26), and who agreed to collect DNA sample, from May 1986 to April 2007. Of the 968 patients, the serum creatinine values at the time of renal biopsy were available for 916 subjects.

### Clinical data

The participating researchers had selected the candidate patients and one qualified nurse, who visited every participated institution, input the clinical data into the formatted database on the website (<http://www.gn.or.kr>) at the time of

renal biopsy and during follow-up visits. During the follow-up period, we gathered the data on age, gender, body weight, height, smoking habit, history of diabetes mellitus, current hypertension, blood pressure, total bilirubin, alanine aminotransferase, aspartate aminotransferase, serum uric acid, serum protein, serum albumin, serum cholesterol, hemoglobin, serum creatinine, proteinuria by dipstick test, urine RBC measured by microscopic examination of urine, medication of angiotensin-converting enzyme inhibitors, angiotensin II type I receptor blockers, other hypertensive agents, any kind of steroid and HMG-CoA reductase inhibitors. Current hypertension was defined as systolic blood pressure of 140 mmHg or more, diastolic blood pressure of 90 mmHg or more, or taking anti-hypertensive medication. Smoking habit was categorized as current smoker or current non-smoker. We calculated the estimated glomerular filtration rate (eGFR) by the modified modification of diet in renal disease (MDRD) equation (29). The renal impairment of IgA nephropathy at biopsy was defined as eGFR less than 60 mL/min/1.73 m<sup>2</sup>.

The mortality and primary cause of death were obtained from the database of the Korean National Statistical Office (<http://www.nso.go.kr>). The mortality data until December 2006 available on this database were searched based on the unique identifier. All study subjects were searched in the national mortality database except 26 patients who were enrolled after January 2007 and 5 patients whose identifiers were not matched to the data of the Korean National Statistical Office. The primary cause of mortality was provided as a 4-digit, ICD-10 code, which was recorded by the physicians. There were 11 deaths during a median follow-up period of 22.0 months (range 0.1-247 months).

### HO-1 genotype assessment

Genomic DNA was isolated from whole blood samples using the a QIAamp blood kit (Qiagen, Valencia, CA, U.S.A.)

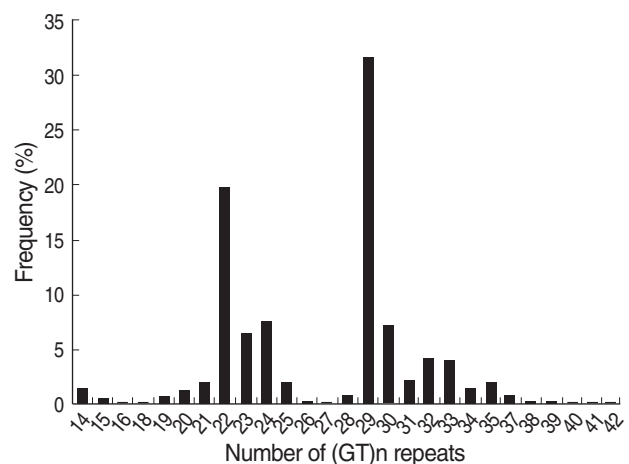


Fig. 1. Frequency distribution of number of (GT) $n$  repeats in all IgA patients.

according to the manufacturer's protocol. Polymerase chain reaction (PCR) amplifications of the HO-1 (GT)<sub>n</sub> repeat length polymorphism were performed as described previously (30). The HO-1 gene 5'-flanking region containing a poly (GT)<sub>n</sub> repeat was amplified by PCR with a 6-FAM-labeled sense primer (5'-AGAGCCTGCAGCTTCTCAGA-3') and an unlabeled antisense primer (5'-ACAAAGTCTCCGGA-TAGGAC-3'), which were designed on the basis of the published sequence (31). Thirty PCR cycles of 94°C for 30 sec, 57°C for 30 sec and 72°C for 30 sec were conducted. For fragment analysis, 1 µL of the PCR product was mixed with 9 µL of HiDi-Formamide (Applied Biosystems, Foster City, CA, U.S.A.) and 0.5 µL of the Genscan 500 LIZ size standard (Applied Biosystems) in 384-well plates. After a denaturation and cooling step, the fragments were analyzed on the ABI 3730XL sequencing system (Applied Biosystems). Each repeat number was calculated with 2 cloned alleles (20, 22) as size markers. For data analysis, we applied GeneMapper version 3.7 software (Applied Biosystems).

### Statistical analysis

The SPSS (SPSS version 12.0, Chicago, IL, U.S.A.) package was used for statistical analysis. Differences in proportions

among the subject groups were compared by chi-square test. Group differences for continuous variables were assessed by the Student t-test or one-way ANOVA test according to the number of groups. To determine whether the genotype group was associated independently with the renal impairment of IgA nephropathy at the time of renal biopsy, we used multiple logistic regression analysis, adjusted for age, gender, and univariate risk factors, for the renal impairment. We repeated the analyses after stratification by gender, age, hypertension, and proteinuria 3+ or more by dipstick test, which are well-known risk factors of renal impairment. We compared the cumulative incidence of mortality by log-rank test. We used Cox's hazard proportional model to determine the relationship between the HO-1 genotype group and mortality. Two-sided *p* values are reported, with the level of statistical significance set at 0.05. All data are shown as mean ± standard deviation or frequency per observation.

## RESULTS

### Allele frequencies at the polymorphic locus

The number of (GT)<sub>n</sub> repeats ranged from 14–42 and exhib-

**Table 1.** Characteristics of patients with IgA nephropathy

	Completeness of data (%)	Genotype groups of HO-1 promoter region*			<i>p</i> value
		S/S (n=66)	S/M, S/L (n=345)	M/M, L/M, L/L (n=505)	
Age (yr)	100.0	33.1 ± 12.8	36.6 ± 13.8	36.1 ± 13.4	0.158
Gender (female %)	100.0	37.9	43.5	46.3	0.368
Habit of smoking (yes %)	92.7	21.9	15.9	12.9	0.126
History of diabetes mellitus (%)	94.1	3.1	3.4	2.5	0.777
Hypertension (%)	90.4	35.5	42.0	41.8	0.613
Body mass index (kg/m <sup>2</sup> )	83.2	24.0 ± 3.9	23.2 ± 3.2	23.6 ± 3.8	0.167
Systolic blood pressure (mmHg)	92.0	125 ± 15	125 ± 17	125 ± 19	0.942
Diastolic blood pressure (mmHg)	92.1	78 ± 10	78 ± 12	76 ± 13	0.641
Total bilirubin (mg/dL)	93.8	0.65 ± 0.38	0.67 ± 0.35	0.65 ± 0.35	0.778
Alanine aminotransaminase (U/L)	96.6	18 ± 10	20 ± 17	20 ± 18	0.685
Aspartate aminotransferase (U/L)	93.2	20 ± 6	20 ± 8	22 ± 17	0.324
Uric acid (mg/dL)	86.7	6.0 ± 1.9	6.0 ± 1.8	6.2 ± 1.9	0.498
Protein (g/dL)	95.3	6.6 ± 0.7	6.6 ± 0.8	6.6 ± 0.9	0.696
Albumin (g/dL)	95.6	3.8 ± 0.6	3.9 ± 0.6	3.8 ± 0.7	0.530
Cholesterol (mg/dL)	91.5	182 ± 53	191 ± 75	190 ± 63	0.682
Hemoglobin (g/dL)	97.7	13.3 ± 2.1	13.0 ± 2.0	12.9 ± 2.0	0.395
Creatinine (mg/dL)	100.0	1.1 ± 0.5	1.3 ± 1.3	1.3 ± 1.0	0.308
Proteinuria 3+ or more* (%)	91.7	25.0	17.9	23.0	0.171
Hematuria* (%)	92.1	95.2	88.5	90.9	0.221
Medication (%)					
ACEI or ARB*	100.0	60.6	59.7	55.0	0.340
Steroids	100.0	10.6	11.9	11.9	0.953
HMG-CoA reductase inhibitor	100.0	12.1	17.4	16.0	0.556

\*Allele S, (GT)<sub>n</sub> repeats less than 23 in HO-1 promoter region; Allele M, (GT)<sub>n</sub> repeats 23–28 in HO-1 promoter region; Allele L, (GT)<sub>n</sub> repeats 29 or more in HO-1 promoter region; hematuria, ≥ 5 RBCs/HPF under light microscopy, proteinuria measured by urine dipstick test; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II type I receptor blocker.

ited a bimodal distribution with one peak located at 22 GT repeats and the other at 29 GT repeats (Fig. 1). Because the inducibility of the HO-1 gene promoter was positively correlated with the number of (GT) repeats (19, 20), we divided allelic repeats into 3 subclasses with short, medium, and long (GT)<sub>n</sub> repeats: short repeats with less than 23 (GT)<sub>n</sub> were designated as allele class S (short), medium repeats with 23 to 28 (GT)<sub>n</sub> as allele class M (medium), and long repeats with 29 or more (GT)<sub>n</sub> as allele class L (long). We had 6 genotypes, S/S, S/M, M/M, S/L, L/M, and L/L, with percentage frequencies (absolute frequencies) of 7.2% (66), 6.9% (63), 3.1% (28), 30.8% (282), 22.7% (208), and 29.4% (269), respectively. The genotypes were categorized into 3 groups: group 1 with S/S genotype, group 2 with S/M and S/L genotypes, and group 3 with M/M, L/M, and L/L genotypes.

### Clinical characteristics

There were no differences in any of the clinical parameters, including medications, among the three genotype groups (Table 1). Group 1 showed no difference in clinical parameters

from either of the other two groups. The bilirubin level was not related to the HO-1 genotypes.

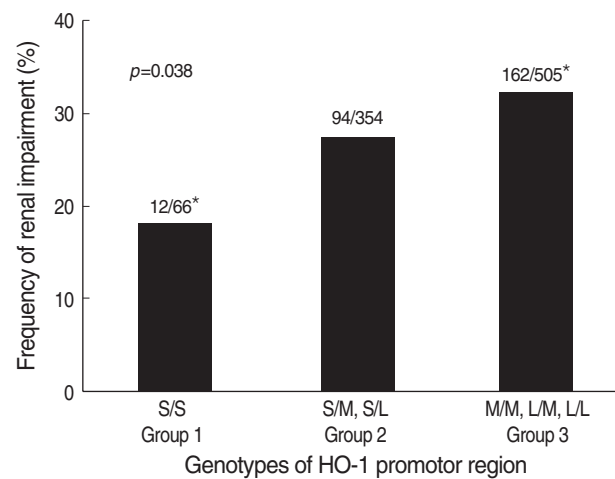


Fig. 2. Frequency of eGFR less than 60 mL/min/1.73 m<sup>2</sup> according to genotypes of the HO-1 promoter region.

\*, compared between genotype group 1 and group 3;  $p=0.021$ .

**Table 2.** Multivariate logistic regression model for the presence of eGFR less than 60 mL/min/1.73 m<sup>2</sup> at diagnosis

Variables	B	Wald	p value	OR [95% C.I.]
<b>Model 1</b>				
HO-1 promoter genotypes*	–	–	0.035	–
S/S	-1.531	5.543	0.019	0.216 [0.060-0.774]
S/M, S/L	-0.379	2.044	0.153	0.685 [0.408-1.151]
Age (yr)	0.074	47.764	<0.001	1.077 [1.054-1.100]
Hypertension (yes)	0.822	10.501	0.001	2.275 [1.384-3.739]
Uric acid (mg/dL)	0.649	68.237	<0.001	1.914 [1.641-2.233]
Hemoglobin (g/L)	-0.570	62.240	<0.001	0.566 [0.491-0.652]
Urine protein by dipstick (≥3+)	0.686	5.672	0.017	1.985 [1.129-3.489]
<b>Model 2</b>				
HO-1 promoter genotypes (S/S) <sup>†</sup>	-1.389	4.676	0.031	0.249 [0.071-0.878]
Age (yr)	0.072	46.503	<0.001	1.075 [1.053-1.098]
Hypertension (yes)	0.836	10.919	0.001	2.307 [1.405-3.788]
Uric acid (mg/dL)	0.648	68.336	<0.001	1.912 [1.640-2.230]
Hemoglobin (g/L)	-0.566	62.007	<0.001	0.568 [0.493-0.654]
Urine protein by dipstick (≥3+)	0.732	6.562	0.010	2.079 [1.187-3.638]
<b>Model 3</b>				
HO-1 promoter genotypes (S/S, M/S, L/S) <sup>‡</sup>	-0.515	4.069	0.044	0.598 [0.362-0.985]
Age (yr)	0.075	49.650	<0.001	1.078 [1.055-1.100]
Hypertension (yes)	0.813	10.383	0.001	2.254 [1.375-3.695]
Uric acid (mg/dL)	0.641	67.213	<0.001	1.898 [1.628-2.212]
Hemoglobin (g/L)	-0.563	62.375	<0.001	0.569 [0.495-0.655]
Urine protein by dipstick (≥3+)	0.641	5.063	0.024	1.899 [1.086-3.320]

Allele S, (GT)<sub>n</sub> repeats less than 23 in HO-1 promoter region; Allele M, (GT)<sub>n</sub> repeats 23-28 in HO-1 promoter region; Allele L, (GT)<sub>n</sub> repeats 29 or more in HO-1 promoter region.

Model 1-3: adjusted with gender and univariate factors to the presence of eGFR less than 60 mL/min/1.73 m<sup>2</sup>, such as age, diabetes mellitus, hypertension, systolic blood pressure, diastolic blood pressure, serum cholesterol, serum uric acid, serum albumin, serum bilirubin, hemoglobin, proteinuria 3+ or more by dipstick test, and genotype of HO-1 promoter region.

\*, compared to M/M, L/M, L/L genotypes (group 3); <sup>†</sup>, compared to S/M, S/L, M/M, L/M, and L/L genotypes (group 2 and group 3); <sup>‡</sup>, compared to M/M, L/M, and L/L genotypes (group 3).

OR, odds ratio; C.I., confidence interval.

**Table 3.** The frequency of eGFR less than 60 mL/min/1.73 m<sup>2</sup> of genotypes of the HO-1 promoter region in each subgroup

	Genotype S/S	Genotype others*	<i>p</i> value
In males	6 (14.6)	132 (28.3)	0.045
In females	6 (24.0)	124 (32.3)	0.377
In younger group aged less than 50 yr	10 (17.5)	168 (23.8)	0.281
In older group aged 50 yr or more	2 (22.2)	88 (60.7)	0.022
In normotensive patients	2 (5.0)	74 (16.6)	0.029
In hypertensive patients	8 (36.4)	153 (47.7)	0.304
In patients with proteinuria less than 3+ by dipstick	6 (13.3)	155 (25.1)	0.059
In patients with proteinuria 3+ or more by dipstick	5 (33.3)	75 (46.0)	0.339

Number, n (%); Genotype S/S (group 1), Patients who had (GT)<sub>n</sub> repeats less than 23 in both alleles of HO-1 promoter region.

\*, Genotype S/M, S/L, M/M, L/M, L/L (group 2 and group 3).

### The HO-1 genotype and renal impairment of IgA nephropathy at the time of renal biopsy

The prevalence of renal impairment differed among the three genotypic groups. In group 1, the renal impairment rate of 18.2% was lower than 32.2% in group 3 (Fig. 2). The univariate factors related to the renal impairment of IgA nephropathy at the time of renal biopsy were age, diabetes mellitus, hypertension, blood pressures, serum cholesterol, serum uric acid, serum albumin, serum bilirubin, hemoglobin, proteinuria 3+ or more by dipstick urine test, and genotype of the HO-1 gene. In multiple logistic regression analysis adjusted for these factors and gender, the HO-1 genotype was an independent factor for renal impairment at diagnosis (Table 2). The risk of renal impairment in patients with single S allele (group 2) was not significantly different from that of group 3 patients but the odds ratio of renal impairment in group 1 was 0.216-fold lower than that in group 3. The odds ratio of impaired renal function in S/S genotype group was 0.249 compared to the other genotypes (group 2 and group 3) and, in S allele group (group 1 and group 2), was 0.598 compared to the other genotypes (group 3).

### The HO-1 genotype and renal impairment in subgroups stratified by risk factors

When we stratified patients by gender, age with the criterion of 50 yr, hypertension, and proteinuria 3+ or more by dipstick urine test, which are related to the renal impairment in IgA nephropathy, the rate of renal impairment in group 1 was lower than that in the other groups (group 2 and group 3), especially in subgroups of men, subjects aged 50 yr or more, and normotensive subjects (Table 3). In subjects with proteinuria by dipstick test less than 3+, group 1 tended to

**Table 4.** The Cox hazard proportional analysis for death in IgA nephropathy

Variables	B	Wald	<i>p</i> value	RR [95% C.I.]
Diabetes mellitus (presence)	4.424	10.925	0.001	83.411 [6.053-1,149.387]
eGFR (mL/min/1.73 m <sup>2</sup> )	-0.045	10.469	0.001	0.956 [0.930-0.982]
Body mass index (kg/m <sup>2</sup> )	-0.546	9.422	0.002	0.579 [0.409-0.821]

Model: adjusted with age, gender, diabetes mellitus, hypertension, body mass index, systolic blood pressure, diastolic blood pressure, serum cholesterol, serum albumin, hemoglobin, hematuria, estimated GFR, and genotype of HO-1 promoter region.

RR, relative risk to death; C.I., confidence interval.

have a lower frequency of renal impairment than the other groups, although the difference was not statistically significant. When we stratified the female group by age, the frequency of renal impairment of group 1 was lower than that of the other groups in females aged 50 yr or more (0/3 vs. 44/72, *p*=0.019).

### The risk factors for mortality in IgA nephropathy

There were no deaths among the 64 patients of group 1 whereas there were 11 deaths in the 821 patients of the other groups (*p*>0.05). All deaths occurred in patients with the L allelic group (11/732). The causes of death were renal failure, nephrotic syndrome, sepsis, heart failure, and subdural hemorrhage. Among univariate risk factors related to mortality, diabetes mellitus, eGFR, and body mass index (BMI) were independent risk factors (Table 4). Three of 24 patients with diabetes mellitus died, as did 8 of the 807 patients without diabetes mellitus (*p*=0.003). There was no mortality in 624 patients with eGFR 60 mL/min/1.73 m<sup>2</sup> or more, 6 deaths out of 196 patients with eGFR 30-59 mL/min/1.73 m<sup>2</sup>, and 5 deaths among 65 patients with eGFR less than 30 mL/min/1.73 m<sup>2</sup> (*p*<0.001). The subgroups with BMI less than 18.5, 18.5-24.9, 25.0-29.9, and 30.0 kg/m<sup>2</sup> or more showed mortality rates of 7.5% (3/40), 0.8% (4/474), 1.0% (2/210), and 0.0% (0/23), respectively. The patients with BMI less than 18.5 kg/m<sup>2</sup> showed significantly higher mortality than those with BMI 18.5 kg/m<sup>2</sup> or more (*p*=0.011).

## DISCUSSION

In this study, we demonstrated that HO-1 gene promoter polymorphism was related to the prevalence of renal impairment at diagnosis, which is an important risk factor for mortality of IgA nephropathy. HO-1 messenger RNA expression and enzyme activity are greater when the (GT) repeat length in the HO-1 gene promoter region is short but the cut-off point of (GT) repeat number related to HO-1 inducibility



has not been defined absolutely. The cut-off criterion of (GT) repeats of the short allele for HO-1 gene length polymorphism has most commonly been <25 (17, 25, 32, 33), but has also included <28 (24), <27 (21-23), and <23 (19, 20). With the transient-transfection assay with HO-1 gene promoter regions containing various numbers of (GT) repeats, the promoter activity of the HO-1 gene was up-regulated in genes with 16 or 20 (GT) repeats but was not in genes with 29 or 38 (GT) repeats (19). The reporter gene expression driven by the HO-1 gene promoter carrying 22 (GT) repeats was about four- and eight-fold greater than that directed by promoters with 26 and 30 (GT) repeats, respectively (20). Therefore, we grouped the HO-1 gene length polymorphism genotypes into 3 groups with the criteria of less than 23, 23 to 28, and 29 or more (GT) repeats.

The short allele of the HO-1 promoter gene was related to the lower rate of renal impairment defined by the estimated GFR less than 60 mL/min/1.73 m<sup>2</sup> as calculated with the modified MDRD equation. Although the MDRD equation was not fully verified to estimate kidney function in the Korean population, we believe that our results are acceptable because the risk of renal impairment defined by the calculated creatinine clearance less than 60 mL/min by the Cockcroft-Gault equation (34) was also 0.073 (95% confidence interval, 0.014-0.378,  $p=0.002$  by multiple logistic regression analysis) in patients with the S/S genotype compared to the risk in patients with the M/M, L/M, or L/L genotype.

Courtney et al. (25) reported that HO-1 (GT)<sub>n</sub> promoter polymorphism was not related with the progression to end stage renal disease in patients with IgA nephropathy. In their study, the dependent variable compared between the genotypic groups was the mean age of entry into the renal replacement therapy program. The mean age of entry into this program might be a surrogate marker for renal survival in genetic renal diseases like autosomal dominant polycystic kidney disease but is not usually accepted as a primary outcome in IgA nephropathy. Although the data at the time of renal biopsy might not be the data at the disease onset point, we should consider the clinical status at the time of renal biopsy and adjust the differences of clinical status at diagnosis between patient groups to estimate the risk of disease progression, statistically.

Although the glomerular injury in IgA nephropathy is usually provoked by IgA-induced mesangial cell activation and complement activation leading to pro-inflammatory and pro-fibrotic phenotype transformation in mesangial cells (26), increased oxidative stress was reported to play some roles in the development and progression of IgA nephropathy (27, 28). Chen et al. (28) observed that the renal infiltration of polymorphonuclear leukocytes, which has a high potential for the production of reactive oxygen species, increased in patients with IgA nephropathy. Advanced oxidation protein products increased in progressed IgA nephropathy compared with in stable IgA nephropathy and was an independent risk

factor to renal outcome of IgA nephropathy (27).

The HO-1 antioxidant effect is mediated by the removal of pro-oxidant heme, generation of biliverdin and bilirubin, coinduction of ferritin, reduction of hydroxyl radical production, and suppression of the pro-oxidant MCP-1 (reviewed in 1). Renal up-regulation of HO-1 attenuates inducible nitric oxide synthase expression and proteinuria in experimental glomerulonephritis (16). For consideration of the pathogenic mechanism of IgA nephropathy and the roles of the HO-1 gene, the relationship between the renal impairment of IgA nephropathy at diagnosis and the inducibility of the HO-1 gene promoter has logical relevance.

The relationship between HO-1 promoter length polymorphism and renal impairment was evident only in males, partially because of the gender-difference of HO-1 inducibility to various stresses. The induction of HO-1 expression and activity was more enhanced in females than in males after trauma and hemorrhagic shock (35) and myocardial ischemia (36). Actually, the S/S genotype was related to a lower rate of renal impairment in females older than 50 yr, which is older than the mean age of menopause in Korean women of 47 yr (37). The HO-1 inducibility was not related to the renal impairment of patients with more severe clinical findings such as hypertension and high proteinuria, suggesting that the influence of the HO-1 gene might be overwhelmed by the clinical risk factors.

This study had several limitations. The devices for measuring serum creatinine were different among institutions. Jaffe's colorimetric method was used to measure the level of serum creatinine in all of the participating institutions, but the device models differed. We did not standardize the results by calibrating the serum creatinine value of each institution to the result of one standard device. The mortality data were from the national mortality database and we could not assess whether or not the primary cause of mortality was correct.

In conclusion, the short allele of HO-1 gene promoter length polymorphism was related to the lower rate of renal impairment in IgA nephropathy at diagnosis, which is an important risk factor for the mortality of IgA nephropathy.

## APPENDIX

Members of The PREMIER group: Institutions that participated in the study (Investigators).

Cheju National University Hospital (Eun Hee Jang), Chonbuk National University Medical School (Won Kim), Chonnam National University Medical School (Nam Ho Kim, Woo Kyun Bae), Chungbuk National University College of Medicine (Hye Young Kim), Chungnam National University College of Medicine (Young-Tai Shin, Kang Wook Lee, Ki-Ryang Na), Daegu Catholic University Medical Center (Ki Sung Ahn), Dankook University Hospital (Jong Tae Cho, Eun Kyeong Lee), Dong-A University College of Medicine

(Ki Hyun Kim, WonSuk An, Seong Eun Kim), Ewha Womans University School of Medicine (Choi Gyu Bog, Seung-Jung Kim), Gachon University of Medicine and Science (Woo Kyung Chung, Hyun Hee Lee, Jaeseok Yang, Sejoong Kim), Gyeongsang National University Hospital (Se-Ho Chang), Hallym University College of Medicine (Jung Woo Noh, Young Ki Lee, Seong Gyun Kim, Jieun Oh, Young Rim Song), Inha University College of Medicine (Moon Jae Kim, Seoung Woo Lee), Inje University College of Medicine (Yeong Hoon Kim, Won Do Park), Keimyung university school of medicine (Hyun Chul Kim, Sung Bae Park), Konkuk University School of Medicine (Kyo-Soon Kim), Korea University Anam Hospital (Won Yong Cho, Hyoung Kyu Kim, Sang-Kyung Jo), Korea University Ansan Hospital (Cha Dae Ryong, Kang Young Sun), Korea University College of Medicine Guro Hospital (Young-Joo Kwon), Kyungpook National University School of Medicine (Yong-Lim Kim, Sun-Hee Park, Chan-Duck Kim), Pochon CHA University College of Medicine (Dong Ho Yang), Pusan National University School of Medicine (Ihm Soo Kwak, Soo Bong Lee, Dong Won Lee, Sang Heon Song, Eun Young Seoung), Seoul Medical Center (Su-Jin Yoon), Seoul National University Bundang Hospital (Dong-Wan Chae, Ki Young Na, Ho Jun Chin), Seoul National University College of Medicine Boramae Medical Center (Chun Soo Lim, Yoon Kyu Oh), Seoul National University Hospital (Kook Hwan Oh, Kwon Wook Joo, Yon-Su Kim, Curie Ahn, Jin Suk Han, Suhnggwon Kim), Seoul National University Hospital Clinical Institute (Hyung Jin Yoon), Sungkyunkwan University School of Medicine (Kyu-Beck Lee), Sungkyunkwan University School of Medicine Samsung Medical Center (Yoon Goo Kim, Jung Eun Lee), Ulsan University College of Medicine, Asan Medical Center (Sang Koo Lee), Yeungnam University College of Medicine (Jun-Young Do, Jong-Won Park, Kyung-Woo Yoon), ordered by alphabet.

## REFERENCES

1. Courtney AE, Maxwell AP. *Heme oxygenase 1: does it have a role in renal cytoprotection?* *Am J Kid Dis* 2008; 51: 678-90.
2. Nakagami T, Toyomura K, Kinoshita T, Morisawa S. *A beneficial role of bile pigments as an endogenous tissue protector: anti-complement effects of biliverdin and conjugated bilirubin.* *Biochim Biophys Acta* 1993; 1158: 189-93.
3. Vachharajani TJ, Work J, Issekutz AC, Granger DN. *Heme oxygenase modulates selectin expression in different regional vascular beds.* *Am J Physiol Heart Circ Physiol* 2000; 278: H1613-7.
4. Otterbein LE, Zuckerbraun BS, Haga M, Liu F, Song R, Usheva A, Stachulak C, Bodyak N, Smith RN, Csizmadia E, Tyagi S, Akamatsu Y, Flavell RJ, Billiar TR, Tzeng E, Bach FH, Choi AM, Soares MP. *Carbon monoxide suppresses arteriosclerotic lesions associated with chronic graft rejection and with balloon injury.* *Nat Med* 2003; 9: 183-90.
5. Gunther L, Berberat PO, Haga M, Brouard S, Smith RN, Soares MP, Bach FH, Tobiasch E. *Carbon monoxide protects pancreatic beta-cells from apoptosis and improves islet function/survival after transplantation.* *Diabetes* 2002; 51: 994-9.
6. Brouard S, Otterbein LE, Anrather J, Anrather J, Tobiasch E, Bach FH, Choi AM, Soares MP. *Carbon monoxide generated by heme oxygenase 1 suppresses endothelial cell apoptosis.* *J Exp Med* 2000; 192: 1015-26.
7. Kanakiriya SK, Croatt AJ, Haggard JJ, Ingelfinger JR, Tang SS, Alam J, Nath KA. *Heme: a novel inducer of MCP-1 through HO-dependent and HO-independent mechanisms.* *Am J Physiol Renal Physiol* 2003; 284: F546-54.
8. Otterbein LE, Bach FH, Alam J, Soares M, Tao Lu H, Wysk M, Davis RJ, Flavell RA, Choi AM. *Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway.* *Nat Med* 2000; 6: 422-8.
9. Inguaggiato P, Gonzalez-Michaca L, Croatt AJ, Haggard JJ, Alam J, Nath KA. *Cellular overexpression of heme oxygenase-1 up-regulates p21 and confers resistance to apoptosis.* *Kidney Int* 2001; 60: 2181-91.
10. Gonzalez-Michaca L, Farrugia G, Croatt AJ, Alam J, Nath KA. *Heme: a determinant of life and death in renal tubular epithelial cells.* *Am J Physiol Renal Physiol* 2004; 286: F370-7.
11. Woo J, Iyer S, Comejo MC, Mori N, Gao L, Sipos I, Maines M, Buelow R. *Stress protein induced immunosuppression: Inhibition of cellular immune effector functions following overexpression of haem oxygenase (HSP 32).* *Transpl Immunol* 1998; 6: 84-93.
12. Nath KA, Balla G, Vercellotti GM, Balla J, Jacob HS, Levitt MD, Rosenberg ME. *Induction of heme oxygenase is a rapid, protective response in rhabdomyolysis in the rat.* *J Clin Invest* 1992; 90: 267-70.
13. Maines MD, Mayer RD, Ewing JF, McCoubrey WK Jr. *Induction of kidney heme oxygenase-1 (HSP 32) mRNA and protein by ischemia/reperfusion: possible role of heme as both promotor of tissue damage and regulator of HSP32.* *J Pharmacol Exp Ther* 1993; 264: 457-62.
14. Shimizu H, Takahashi T, Suzuki T, Yamasaki A, Fujiwara T, Odaka Y, Hirakawa M, Fujita H, Akagi R. *Protective effect of heme oxygenase induction in ischemic acute renal failure.* *Crit Care Med* 2000; 28: 809-17.
15. Mosley K, Wembridge DE, Cattell V, Cook HT. *Heme oxygenase is induced in nephrotoxic nephritis and hemin, a stimulator of heme oxygenase synthesis, ameliorates disease.* *Kidney Int* 1998; 53: 672-8.
16. Datta PK, Koukouritaki SB, Hopp KA, Lianos EA. *Heme oxygenase-1 induction attenuates inducible nitric oxide synthase expression and proteinuria in glomerulonephritis.* *J Am Soc Nephrol* 1999; 10: 2540-50.
17. Exner M, Bohmig GA, Schillinger M, Regele H, Watschinger B, Horl WH, Raith M, Mannhalter C, Wagner OF. *Donor heme oxygenase-1 genotype is associated with renal allograft function.* *Transplantation* 2004; 77: 538-42.
18. Yachie A, Niida Y, Wada T, Igarashi N, Kaneda H, Toma T, Ohta K, Kasahara Y, Koizumi S. *Oxidative stress causes enhanced endothelial injury in human heme oxygenase-1 deficiency.* *J Clin Invest* 1999;

- 103: 129-35.
19. Yamada N, Yamaya M, Okinaga S, Nakayama K, Sekizawa K, Shibahara S, Sasaki H. *Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema*. *Am J Hum Genet* 2000; 66: 187-95.
  20. Chen YH, Lin SJ, Lin MW, Tsai HL, Kuo SS, Chen JW, Chang MJ, Wu TC, Chen LC, Ding YA, Pan WH, Jou YS, Chau LY. *Microsatellite polymorphism in promoter of heme oxygenase-1 gene is associated with susceptibility to coronary artery disease in type 2 diabetic patients*. *Hum Genet* 2002; 111: 1-8.
  21. Hirai H, Kubo H, Yamaya M, Nakayama K, Numasaki M, Kobayashi S, Suzuki S, Shibahara S, Sasaki H. *Microsatellite polymorphism in heme oxygenase-1 gene promoter is associated with susceptibility to oxidant-induced apoptosis in lymphoblastoid cell lines*. *Blood* 2003; 102: 1619-21.
  22. Kaneda H, Ohno M, Taguchi J, Togo M, Hashimoto H, Ogasawara K, Aizawa T, Ishizaka N, Nagai R. *Heme oxygenase-1 gene promoter polymorphism is associated with coronary artery disease in Japanese patients with coronary risk factors*. *Arterioscler Thromb Vasc Biol* 2002; 22: 1680-5.
  23. Kikuchi A, Yamaya M, Suzuki S, Yasuda H, Kubo H, Nakayama K, Handa M, Sasaki T, Shibahara S, Sekizawa K, Sasaki H. *Association of susceptibility to the development of lung adenocarcinoma with the heme oxygenase-1 gene promoter polymorphism*. *Hum Genet* 2005; 116: 354-60.
  24. Baan C, Peeters A, Lemos F, Uitterlinden A, Doxiadis I, Claas F, Ijzermans J, Roodnat J, Weimar W. *Fundamental role for HO-1 in the self-protection of renal allografts*. *Am J Transplant* 2004; 4: 811-8.
  25. Courtney AE, McNamee PT, Heggarty S, Middleton D, Maxwell AP. *Association of functional haem oxygenase-1 gene promoter polymorphism with polycystic kidney disease and IgA nephropathy*. *Nephrol Dial Transplant* 2008; 23: 608-11.
  26. Barratt J, Feehally J. *IgA nephropathy*. *J Am Soc Nephrol* 2005; 16: 2088-97.
  27. Descamps-Latscha B, Witko-Sarsat V, Nguyen-Khoa T, Nguyen AT, Gausson V, Mothu N, Cardoso C, Noel LH, Guerin AP, London GM, Jungers P. *Early detection of IgA nephropathy progression: proteinuria and AOPP are strong prognostic markers*. *Kidney Int* 2004; 66: 1606-12.
  28. Chen HC, Tomino Y, Yaguchi Y, Fukui M, Yokoyama K, Watanabe A, Koide H. *Oxidative metabolism of polymorphonuclear leukocytes (PMN) in patients with IgA nephropathy*. *J Clin Lab Anal* 1992; 6: 35-9.
  29. Levey AS, Eckardt KU, Tsukamoto Y, Levin A, Coresh J, Rossert J, De Zeeuw D, Hostetter TH, Lameire N, Eknoyan G. *Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO)*. *Kidney Int* 2005; 67: 2089-100.
  30. Kaneda H, Ohno M, Taguchi J, Togo M, Hashimoto H, Ogasawara K, Aizawa T, Ishizaka N, Nagai R. *Heme Oxygenase-1 Gene promoter polymorphism is associated with coronary artery disease in Japanese patients with coronary risk factors*. *Arterioscler Thromb Vasc Biol* 2002; 22: 1680-5.
  31. Verma A, Hirsch DJ, Glatt CE, Ronnett GV, Snyder SH. *Carbon monoxide: a putative neural messenger*. *Science* 1993; 259: 381-4.
  32. Funk M, Endler G, Schillinger M, Mustafa S, Hsieh K, Exner M, Lalouschek W, Mannhalter C, Wagner O. *The effect of a promoter polymorphism in the heme oxygenase-1 gene on the risk of ischaemic cerebrovascular events. The influence of other vascular risk factors*. *Thromb Res* 2004; 113: 217-23.
  33. Wijkema JS, van Haelst PL, Monraats PS, Bruinenberg M, Zwinderman AH, Zijlstra F, van der Steege G, de Winter RJ, Doevendans PA, Waltenberger J, Jukema JW, Tio RA. *Restenosis after percutaneous coronary intervention is associated with the angiotensin-II type-1 receptor 1166A/C polymorphism but not with polymorphisms of angiotensin-converting enzyme, angiotensin-II receptor, angiotensinogen or heme oxygenase-1*. *Pharmacogenet Genomics* 2006; 16: 331-7.
  34. Cockcroft DW, Gault MH. *Prediction of creatinine clearance from serum creatinine*. *Nephron* 1976; 16: 31-41.
  35. Toth B, Yokoyama Y, Kuebler JF, Schwacha MG, Rue LW 3rd, Bland KI, Chaudry IH. *Sex differences in hepatic heme oxygenase expression and activity following trauma and hemorrhagic shock*. *Arch Surg* 2003; 138: 1375-82.
  36. Zampino M, Yuzhakova M, Hansen J, McKinney RD, Goldspink PH, Geenen DL, Buttrick PM. *Sex-related dimorphic response of HIF-1 alpha expression in myocardial ischemia*. *Am J Physiol Heart Circ Physiol* 2006; 291: H957-64.
  37. Hong JS, Yi SW, Kang HC, Jee SH, Kang HG, Bayasgalan G, Ohrr H. *Age at menopause and cause-specific mortality in South Korean Women: Kangwha Cohort Study*. *Maturitas* 2007; 56: 411-9.